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     (FILE 'USPAT' ENTERED AT 15:16:28 ON 30 JUN 1999)
L1          5297 S CYTOKINE?
L2          6622 S INTERLEUKIN?
L3          6860 S INTERFERON?
L4          3013 S LYMPHOKINE?
L5           518 S LYMPHOTOXIN?
L6          3165 S TUMOR NECROSIS
L7            57 S TARGET MOIET?
L8         59941 S ANTIBOD? OR RECEPTOR?
L9           106 S L1 AND L2 AND L3 AND L4 AND L5
L10           94 S L9 AND L6
L11            0 S L10 AND L7
L12           92 S L10 AND L8
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US PAT NO: 5,587,294 [IMAGE AVAILABLE] L12: 56 of 92

ABSTRACT:
The present invention relates to methods for measuring endogenous **cytokines** in blood. The method accurately measures the **cytokines** in the blood in the presence of substances that bind the **cytokines** thereby causing the measurement of the **cytokines** by conventional methods to give inaccurate results. The present invention also includes the non-invasive measurement of **cytokines** in biological fluids such as saliva and nasal secretions. Finally, the present invention allows one to monitor the level of **cytokines** in the blood during treatment of a human or animal with **cytokines**.

US PAT NO: 5,587,294 [IMAGE AVAILABLE] L12: 56 of 92
TITLE: Method and kit for measuring endogenous **cytokines**

ABSTRACT:
The present invention relates to methods for measuring endogenous **cytokines** in blood. The method accurately measures the **cytokines** in the blood in the presence of substances that bind the **cytokines** thereby causing the measurement of the **cytokines** by conventional methods to give inaccurate results. The present invention also includes the non-invasive measurement of **cytokines** in biological fluids such as saliva and nasal secretions. Finally, the present invention allows one to monitor the level of **cytokines** in the blood during treatment of a human or animal with **cytokines**.

SUMMARY:

BSUM(2)

The . . . monitoring immunological function in a human or animal. More particularly, the present invention relates to accurately measuring the concentration of **cytokines** in the blood and other body fluids, including but not limited to, saliva, nasal secretions, tears and sweat.

SUMMARY:

BSUM(4)

As used herein, the term "**cytokine**" is defined as growth factors secreted by immune or other cells, whose action is on cells of the immune system, such as, but not limited to, T-cells, B-cells, NK cells and macrophages. Representative **cytokines** include, but are not limited to, the group consisting of **interleukin** 1.alpha., **interleukin**-1.beta., **interleukin**-2, **interleukin**-6, **interferon**-alpha, **interferon**-gamma, **tumor necrosis** factor-.alpha., growth factors, such as TGFB, NGF, EGF, and oncogenes such as c-myc and c-fos. The term "EIA" means any immunoassay utilizing enzymes as the label. The term "endogenous **cytokines**" as used herein, means **cytokines** that are produced in vivo and normally circulate in the blood and various other biological fluids. The term includes prohormones which are larger molecular weight forms of **cytokines** which have not yet undergone post-transcriptional modification.

SUMMARY:

BSUM(10)

A . . . above. One reason this assay has become popular is the relative ease of its performance compared to most antigen-specific or **antibody**-dependent cellular cytotoxicity assays. However, compared to immunoassays commonly used for endocrine hormones, it still ranks as a rather difficult bioassay. . .

SUMMARY:

BSUM(12)

A . . . to monitoring the immune system that has grown in popularity

is the enumeration of lymphocyte subsets by flow cytometry using **antibodies** specific for cell-surface markers (Ault, K., MANUAL OF CLINICAL LABORATORY IMMUNOLOGY (3rd Ed.), Rose, N. R., et al., (eds.), 247-253. . .

SUMMARY:

BSUM(13)

A . . . 3d et al., LANCET 1:1400-1402 (1983)). The total concentration of salivary IgA reflects the presence of a large collection of **antibodies** of unknown antigen specificity. Furthermore, it is not clear how total IgA levels in saliva relate to the overall dynamics of the immune system. IgA **antibodies** are associated with mucosal surfaces and are thought to protect these surfaces from infection. Consequently, secretory IgA is found not. . .

SUMMARY:

BSUM(14)

Importantly, . . . physiological changes which could contaminate the data obtained. Thus, a method which would allow measurement of salivary levels of a **cytokine** or other product of the immune system would have several major advantages over existing approaches, such as (a) eliminating the. . .

SUMMARY:

BSUM(15)

Two regulatory molecules of the immune system are **interleukin** 1 and 2 (IL-1 and IL-2). The ability of IL-1 and IL-2 to modulate a **cytokine** "cascade" and the concomitant cell proliferation, differentiation and effector function of lymphoid cells has been characterized in detail (see, for. . .

SUMMARY:

BSUM(16)

These . . . molecular signals are apparently not limited to action within the immune system, as recent studies indicate that both of these **cytokines** act as homeostatic regulators outside the immune system. For example, IL-1 has been shown to act as a potential modulator. . . et al., ANTICANCER RES. 8:1233-1240 (1988); Paciotti, G. F. et al., ANTICANCER RES. 11:25-32 (1991)). It appears, therefore, that these **cytokines** not only affect classical autocrine/paracrine loops within the immune system, but also affect endocrine circuits and may therefore modulate interactions. . .

SUMMARY:

BSUM(17)

For . . . a quantitative manner, the endogenous concentrations of these molecules. Conventional methods of measuring IL-1 and IL2, as well as other **cytokines**, directly in the blood have been unsatisfactory.

SUMMARY:

BSUM(18)

To . . . in the adaptive immune response and other forms of host defense. As a result of this focus, study of these **cytokines** has been

limited to stimulation of cells cultured in vitro, or the measurement of blood **cytokine** levels in vivo in immunological diseases. Recent studies indicate that IL-1 should be considered an "endogenous" component of the circulation;. . .

SUMMARY:

BSUM(19)

Generally, in viewing IL-1 and IL-2 or other **cytokines** as measures of immunocompetence in vivo, investigators have focused on large changes in concentration under severe pathophysiological conditions such as leukemia and arthritis. Furthermore, reports of circulating **cytokine** levels have been concerned with the elevation in patients compared with controls, while paying little attention to the fact that. . . variety of subclinical. factors (Michie, H. R. et al., NEW ENG. J. MED. 318:1481-1486 (1988); Grau, G. E. et al., **LYMPHOKINE** RES. 7:335 (1988); Shenkin, A. et al., **LYMPHOKINE** RES. 7,333 (1988)).

SUMMARY:

BSUM(20)

In addition to measurement of **cytokines** in serum or plasma, various **cytokines** have been detected in other biological fluids. For example, Kimball, E. C. et al., (J. IMMUNOL. 133:256-260 (1984)) reported IL-1. . . methods. The same group used enzyme immunoassays to measure IL-1.alpha. and IL-1.beta. in human amniotic fluid (Tsunoda, H. et al., **LYMPHOKINE** RES. 7:333 (1988)). Wilmott, R. W. et al., (**LYMPHOKINE** RES. 7:334 (1988)) measured IL-1.beta. (by EIA) and IL-1 bioactivity in human bronchoalveolar lavage fluid in cystic fibrosis compared to. . . (MOL. CELL. ENDOCRINOL. 58:221-230 (1988)) reported that high levels of IL-1-like bioactivity could be demonstrated in human ovarian follicular fluid. **Lymphotoxins** have been reported in blister fluid of pemphigoid patients (Jeffes, E. W. et al., J. CLIN. IMMUNOL. 4:31-35 (1984)). IL-1 has also been reported in human sweat (Didierjean, et al., "Biologically active **interleukin** 1 in human eccrine sweat: Site dependent variations in .alpha./.beta. ratios and stress-induced increased secretion," **CYTOKINE** 2:438-446 (1990)).

SUMMARY:

BSUM(21)

IL-1 . . . (see, for example, Peter, J. B. et al., NEUROLOGY 41:121-123 (Jan 1991)). When Peter et al. (supra) examined IL-1.beta. and **tumor necrosis** factor (TNF) in CSF and serum of multiple sclerosis patients and normal controls, they concluded that the levels of these **cytokines** in these two fluids were not of prognostic or diagnostic utility. Westacott, C. I. et al., (ANN. RHEUM. DIS. 49:676-681 (1990)) used immunoassays to measure **cytokines** in synovial fluid of patients with rheumatic disease (EIA for IL-1.beta.; RIA for IL-2, TNF, IFN alpha and gamma).

SUMMARY:

BSUM(23)

As can be seen from the foregoing overview of the literature, there have been many attempts to measure endogenous **cytokines** in blood and other body fluids. However, in reviewing these reports, it is apparent that there is wide variation in the reported results with regard to **cytokine** concentration in the blood and to fluctuations of **cytokine** concentration in the blood.

SUMMARY:

BSUM(24)

Many reports indicate that **cytokines** (i.e., IL-2) are not detectable in normal subjects using immunoassays. It is possible that circulating IL-2 may be bound by the well-described soluble IL-2 **receptor**. The site of attachment may interfere with recognition by the capture **antibody** of a sandwich assay system, which would make IL-2 appear undetectable. Alternatively, even if the molecule is captured, the detection by the second **antibody** may be prevented by steric hindrance by the binding of both the capture **antibody** and the soluble IL-2 **receptor** to IL-2. In effect, there may not be sufficient space to permit the binding of yet a third large protein.. . .

SUMMARY:

BSUM(25)

It is possible that some assay procedures detect very little **cytokine**, whereas others pickup none at all. This difference may be related to the assay system or to the **cytokine** or both. The problem has been reported by the observations of Cannon, J. G., et al., **LYMPHOKINE** RES., 7:457-465 (1988), in which the authors show that some plasma substance inhibited the assay, effecting detection. In this study,. . . clear from this study if the plasma factors simply effect the performance of the assay or are related to the **cytokine** itself. This question was further described by Capper, S. J., et al., **CYTOKINE** 2:182-189 (1990). Capper, S. J., et al. show that IL-1 .alpha. and .beta. are bound by proteins and that the. . .

SUMMARY:

BSUM(26)

The best example of a **cytokine** "binding protein" may be inferred from the data describing a soluble IL-2 **receptor** found in the circulation. This molecule has been shown to be immunologically similar to the low affinity IL-2 **receptor** on T-cells, "Tac". Its presence in the circulation, free of the T-cell, would strongly argue that it can no longer be a **receptor** capable of transmitting signals from outside the cell to inside the cell. However, these data do not address the ability. . . by this protein, and it is this bound complex which essentially makes the endogenous IL-2 undetectable by "sandwich" assays. Other **cytokines** (e.g., IL-1) may be bound to other carrier molecules in serum that effectively masks their detection.

SUMMARY:

BSUM(27)

In addition, while various **cytokines**, including IL-1, have been reported in certain normal or pathological biological fluids, there have been no reports of **cytokines** or **lymphokines** in saliva or nasal secretions. In fact, a paper reporting a study of the pharmacodynamics of **interferon** (Diez, R. A. et al., J. **INTERFERON** RES. 7:553-557 (October 1987)) stated that ". . . at present, whether **interferon** is present in saliva and nasal secretion is unclear."

SUMMARY:

BSUM(28)

It would be of great benefit if one could easily, accurately and reproducibly measure the concentrations of various endogenous

cytokines in the body fluids. This would create a useful window not only into the immune system but into a myriad. . .

SUMMARY:

BSUM(29)

What is needed is a reliable method of measuring endogenous **cytokines** in blood which will result in an accurate blood concentration of the **cytokine** independent of binding proteins which may be bound to the circulating **cytokine**. In addition, ability to measure **cytokines** in a biological fluid such as saliva or nasal secretions would simplify the analysis of an individual's immune system, possibly. . .

SUMMARY:

BSUM(31)

The present invention is a competitive solid phase immunoassay for use in detecting and monitoring endogenous **cytokines** in humans or animals. The competitive solid phase immunoassay is a "one site" immunoassay rather than a "sandwich" assay. The present invention is especially useful for measuring endogenous **cytokine** levels in the blood and other biological fluids. Prior art methods have been unable to reliably measure **cytokine** levels in the blood because **cytokine**-binding proteins (or other blood products) appear to mask the **cytokine** protein. By measuring the **cytokines** using the present invention, the concentration of a particular **cytokine** can be accurately determined. In addition, the present invention is particularly useful in measuring endogenous **cytokines** in fluids such as saliva, nasal secretions, and tears.

SUMMARY:

BSUM(32)

The present invention provides the capability of easily sampling body fluids and thereby measuring "normal" as well as "stimulated" levels of **cytokines**. The present invention provides a new tool for monitoring these chemical communication signals and their dysregulation in the face of. . .

SUMMARY:

BSUM(33)

Thus, the present invention is also directed to a method for the non-invasive determination of the level of a **cytokine** in a human or animal, comprising measuring the concentration of the **cytokine** in the saliva or nasal secretion of the human or animal utilizing a novel competitive immunoassay. The immunoassay can be. . .

SUMMARY:

BSUM(34)

The . . . in the blood and other body fluids and includes, but is not limited to, proteins from the group consisting of **interleukin**-1.alpha. **interleukin**-1.beta., **interleukin**-2, **interleukin**-6, **interferon**-alpha, **interferon**-gamma and **tumor necrosis** factor-alpha. It is contemplated that the present invention will be useful in detecting and quantifying other **cytokine**-like molecules in the blood that have not yet been characterized.

SUMMARY:

BSUM(35)

In . . . monitoring immunological activity of a subject comprising measuring in a non-blood body fluid of the subject the concentration of a **cytokine**. In a preferred embodiment, the body fluid is saliva. In another embodiment, the body fluid is nasal secretion.

SUMMARY:

BSUM(36)

Accordingly, it is an object of the present invention to provide a method for accurately measuring the concentration of endogenous **cytokines** in body fluids of humans or animals.

SUMMARY:

BSUM(37)

It is yet another object of the present invention to provide a method for accurately measuring the concentration of endogenous **cytokines** in blood of humans or animals.

SUMMARY:

BSUM(38)

Another object of the present invention is to provide a method for accurately measuring the concentration of endogenous **cytokines** in saliva and nasal secretions.

SUMMARY:

BSUM(39)

Yet another object of the present invention is to provide a method by which the concentration of **cytokines** in a body fluid can be correlated to a pathological condition.

SUMMARY:

BSUM(40)

Yet another object of the present invention is to provide a method for measuring **cytokines**.

SUMMARY:

BSUM(41)

It is another object of the present invention to provide a method for monitoring **cytokine** levels which does not require a clinical setting for conducting the tests.

SUMMARY:

BSUM(42)

It is yet another object of the present invention to provide a method for evaluating and measuring **cytokine** levels in response to behavioral perturbations.

SUMMARY:

BSUM(43)

It is a further object of the present invention to provide a method for evaluating **cytokine** levels as a response to chemical, viral or bacterial challenges.

SUMMARY:

BSUM(44)

It is yet another object of the present invention to provide a method for monitoring **cytokine** levels during the course of an identified disease.

SUMMARY:

BSUM(45)

It is an object of the present invention to provide a method for using measurements of **cytokine** levels as an index of the risk of disease.

SUMMARY:

BSUM(46)

It is another object of the present invention to provide a method for using **cytokine** levels as an indicator of clinical flare-ups.

DRAWING DESC:

DRWD(2)

FIG. 1 is a graph showing relative **antibody** titer of rabbit polyclonal antiserum to recombinant human **interleukin**-1.alpha..

DRAWING DESC:

DRWD(8)

FIG. 7 is a graph showing HPLC validation of fractionated **cytokine**-spiked serum treated with **antibodies** specific for IL-1 (FIG. 7A) or IL-2 (FIG. 7B).

DETDESC:

DETD(2)

The present invention provides methods for measuring the level of a **cytokine** in a biological fluid from a human or animal such as blood, saliva, nasal secretions or tears. According to the present invention, **cytokine**-type proteins can be accurately measured in the blood even in the presence of **cytokine** binding proteins which mask the **cytokine** activity in conventional assays.

DETDESC:

DETD(3)

These methods are particularly useful for monitoring immunological activity in a subject. Such monitoring may be used in (1) subjects undergoing **cytokine** immunotherapy, or other forms of therapy, (2) patients with immunological disorders in which salivary **lymphokine** or **cytokine** levels are abnormal, (3) individuals being studied for the effects of behavioral influences on immune function, and the like. The.

. . may be used to detect the presence or measure the concentration or level of any of a number of known **cytokines** or **lymphokines**, including, but not limited to, IL-1, IL-2, IL-4, IL-6, IFN-alpha, IFN-gamma, TNF- alpha and TNF-beta.

DETDESC:

DETD(4)

The present inventors have developed competitive EIAs which are capable of being used to accurately measure **cytokines** in serum as well as saliva and nasal secretions.

DETDESC:

DETD(5)

Being able to measure **cytokines** in saliva and nasal secretions allows one to measure the proteins in more easily obtainable body fluids than blood.

DETDESC:

DETD(6)

The present invention includes any one-site assay system that is preferably polyclonal **antibody**-based. It has been discovered by the present inventors that even though a **cytokine** may be bound to another molecule in the biological fluid, there is at least be one part of the molecule that is available for site recognition. This site is accessible to polyclonal **antibody** binding, making it detectable in the one site system.

DETDESC:

DETD(7)

This . . . and levels of IL-2 were then detectable. These data suggested that the two-site assay would be problematic, a one-site polyclonal **antibody** system was developed. This one-site system is capable of detecting IL-2 in normal serum or plasma without further processing. In. . .

DETDESC:

DETD(9)

The method of the present invention for measuring the level of a **cytokine** in a biological fluid typically comprises incubating the biological fluid in the presence of an **antibody** capable of binding to the **cytokine** and detecting the amount of the **cytokine** bound or not bound to the **antibody**.

DETDESC:

DETD(11)

The . . . (1986)) to measure hormones and growth factors in biological matrices. The manner by which an unknown ligand, such as a **cytokine**, is detected is similar to that of a competitive radioimmunoassay. Briefly, a specific amount of a labeled analyte, for example,. . . is in competition with unlabeled IL-1 (either in the unknown sample or in a standard) for a limited number of **antibody** binding sites.

DETDESC:

DETD(12)

The . . . subject wherein the measurement is performed on more than one occasion. Furthermore, the above method provides a method for monitoring **cytokine** therapy in a subject wherein the subject being monitored is one undergoing **cytokine** therapy. Preferably, the **cytokine** being monitored is the **cytokine** of the immunotherapy.

DETDESC:

DETD(13)

In a preferred embodiment of the present invention, in the first step of the assay, an **antibody**, preferably a rabbit polyclonal **antibody** which recognizes many epitopes on the **cytokine** molecule, is adsorbed to a solid phase support or carrier, preferably the wells of a polystyrene EIA plate. This **antibody**, known as a "capture **antibody**," is then used to bind the labeled analyte, e.g. biotinylated IL-1, and the unlabeled analyte in the sample or the standard. After appropriate washing steps, an enzyme-conjugated binding partner for the label, for example, streptavidin or an anti-biotin **antibody**, is incubated with the **antibody**-analyte complex, allowing the enzyme to be bound to the complex. After removal of any unbound enzyme-conjugated binding partner, a chromogenic. . . in the sample competed successfully with the fixed amount of biotinylated IL-1 for binding to a fixed amount of immobilized **antibody**, and bound non-labeled IL-1 does not result in subsequent binding of the binding partner-enzyme complex.

DETDESC:

DETD(14)

The . . . being used as an overnight assay. In a preferred form of the assay, during the first two hours, the capture **antibody**, preferably a rabbit anti-human **cytokine antibody**, is adsorbed to the wells of a 96-well immunoplate. During the next two hours, unbound **antibody** is washed off the plate followed by addition of either standards or unknowns, as well as a specific amount of labeled **cytokine**, preferably biotinylated **cytokine**. The amount of labeled **cytokine** which has bound is then detected by the addition of the binding partner, preferably streptavidin, conjugated to an enzyme, preferably. . .

DETDESC:

DETD(15)

The term "solid phase support" means any support capable of binding antigen or **antibodies**. Well-known supports, or carriers, include, but are not limited to, polystyrene, polypropylene, polyethylene, glass, dextran, nylon, amylases, natural and modified. . . The support material may have virtually any structural configuration so long as the antigen is capable of binding to an **antibody**. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a. . . sides of a polystyrene microtiter plate well. Those skilled in the art will know many other suitable carriers for binding **antibody** or antigen, or will be able to ascertain the same by use of routine experimentation.

DETDESC:

DETD(16)

A preferred means of labeling the analyte, e.g., the **cytokine** (or anti-**cytokine antibody**, as discussed below) is by linking to it to label which can be bound to a binding partner which is. . .

DETDESC:

DETD(17)

It is also possible to label the analyte (or **antibody**) with a fluorescent compound. When the fluorescent labeled bound analyte is exposed to light of the proper wave length, its. . .

DETDESC:

DETD(18)

The analyte or **antibody** can also be labeled with fluorescence emitting metals such as .sup.152 Eu, or others of the lanthanide series. These metals can be attached to the analyte or **antibody** using such metal chelating groups as diethylene-triaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

DETDESC:

DETD(19)

The analyte or **antibody** also can be labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged bound molecule is then. . .

DETDESC:

DETD(20)

Likewise, a bioluminescent compound may be used to label the analyte or **antibody**. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the. . .

DETDESC:

DETD(21)

In addition to use of the enzyme immunoassay, the method of the present invention can measure the level of a **cytokine** using any of a variety of other immunoassays. For example, by radioactively labeling the **cytokine** (or the **cytokine**-specific **antibodies** or **antibody** fragments as described below), it is possible to detect the **cytokine** through the use of a radioimmunoassay (RIA). A good description of RIA may be found in Weintraub, B., PRINCIPLES OF. . .

DETDESC:

DETD(23)

The binding activity of a given lot of anti-**cytokine antibody** may be determined according to well known methods. Those skilled in the art will be able to determine operative and. . .

DETDESC:

DETD(25)

Detection of the labeled **antibody** or binding partner for the labeled

analyte may be accomplished by a scintillation counter, for example, if the detectable label. . .

DETDESC:

DETD(28)

In . . . preferably saliva. The device is installed in the mouth of a subject where it accumulates compounds of interest, preferably a **cytokine** as described herein, by passive diffusion. The device is a small, sealed plastic cylinder with a plurality of tiny ports. . . within the inner chamber of the device a substance which can bind the substance to be measured, for example an **antibody** specific for the **cytokine**. More recently, the utility of such a device for the time-integrated measurements of corticosteroids in human saliva has been demonstrated. . .

DETDESC:

DETD(29)

The . . . kits for use with the methods of the present invention. The kit of the present invention is useful for measuring **cytokines** in a body fluid, preferably saliva or nasal secretions. Each kit contains detailed instructions on the collection of the biological. . .

DETDESC:

DETD(32)
(b) a second container containing a first binding partner specific for the **cytokine** to be measured;

DETDESC:

DETD(33)
(c) a third container containing purified **cytokine** in labeled form; and

DETDESC:

DETD(34)
(d) a fourth container containing a second binding partner for said label on said labeled **cytokine**.

DETDESC:

DETD(40)
(1) a capture **antibody** specific for the **cytokine** to be measured;

DETDESC:

DETD(41)
(2) purified **cytokine** in labeled form;

DETDESC:

DETD(42)
(3) standard human **cytokine** to serve as the assay standard; and

DETDESC:

DETD(43)
(4) enzyme conjugated binding partner for the label on the labeled **cytokine**.

DETDESC:

DETD(52)
(1) purified polyclonal rabbit anti-human IL-1.alpha. **antibody** (the capture **antibody**);

DETDESC:

DETD(63)

For example, there may be a container containing the capture **antibody** in fluid phase or alternatively, already immobilized on a solid phase support. A further container contains labeled (e.g., biotin- or enzyme-conjugated) **cytokine**, or labeled **antibodies** in solution. Further containers may contain standards comprising serial dilutions of the **cytokine** to be detected. The standard solutions of the **cytokine** are used to prepare a standard curve with the concentration of the **cytokine** plotted on the abscissa and the detection signal on the ordinate. The results obtained from a sample, e.g. saliva, containing the **cytokine** may be interpolated from such a plot to give the concentration of the **cytokine**.

DETDESC:

DETD(64)

In the above kit, the **cytokine** is preferably selected from the group consisting of **interleukin**-1.alpha., **interleukin**-1.beta., **interleukin**-2, **interleukin**-6, **interferon**-alpha, **interferon**-gamma and **tumor necrosis** factor-alpha. Most preferably, the **cytokine** is **interleukin** 1.alpha., **interleukin**-1.beta., or **interleukin**-2.

DETDESC:

DETD(65)

In one embodiment of the above kit, a preferred label for the **cytokine** is biotin and a preferred second binding partner is streptavidin. In a preferred embodiment, the first binding partner is a capture **antibody** specific for the **cytokine**, the second binding partner is an enzyme-conjugated binding partner, preferably enzyme conjugated-streptavidin. Preferably, the enzyme is alkaline phosphatase. The kit. . .

DETDESC:

DETD(67)

The . . . immune system of humans, such as researchers interested in brain-immune system interactions or physicians following a patient being treated with **cytokines**, agents that affect **cytokine** levels or agents that act upon the immune system and whose action may be reflected as altered **cytokine** concentrations in a body fluid, such as saliva.

DETDESC:

DETD(70)
Generation and Purification of Polyclonal **Antibodies** Specific for **Interleukin**-1.alpha. and **Interleukin**-2

DETDESC:

DETD(71)

Pathogen-free . . . after this injection, five ml of blood is drawn through an ear vein and the resultant sera is tested for **antibody** titers (described below). Approximately two weeks after the second injection, each rabbit is boosted with only the peptide/enhancer mixture and. . .

DETDESC:

DETD(73)

After . . . of either IL-1 or IL-2 into a rabbits, a five ml blood sample is drawn and the serum tested for **antibody** titer. FIG. 1a demonstrates that the immunogenic enhancer facilitated the generation of very high titer antiserum specific for IL-1.alpha.. As. . .

DETDESC:

DETD(74)

Each . . . antiserum is purified by column chromatography using a mixed ion exchange resin (J. T. Baker, Inc., Phillipsburg, N.J.). The resin-bound **antibody** is eluted from the column using a linear gradient of 0 to 0.75 M NaCl in 25 mM MES (2-[N-Morpholino]ethanesulfonic. . . M NaCl). Five ml fractions are collected and analyzed for protein content (absorption at 280 nm). The presence of specific **antibodies** is tested by a direct enzyme immunoassay (EIA). Rabbit **antibodies** are detected by an alkaline phosphatase-conjugated goat anti-rabbit **antibody**. Those fractions which result in a signal-to-noise ratio of five or more are pooled and dialyzed against PBS. The resultant pooled aliquots serve as the **antibody** solution for their respective EIAs.

DETDESC:

DETD(79)

The test bleeds for either peptide and all subsequent production bleeds are tested for relative **antibody** tilers by a direct EIA. Either IL-1 or IL-2 is diluted in coating buffer (15.9 g/L Na.sub.2 CO.sub.3, 29.3 g/l. . .

DETDESC:

DETD(82)

Purified . . . for an additional 1 hour. The wells are then washed and incubated for 45 minutes with alkaline phosphatase conjugated anti-rabbit **antibody** (Assay Research, Inc.) followed by the addition of substrate. The resultant color reaction is determined at an absorbance of 405. . .

DETDESC:

DETD(83)

FIGS. . . . 3 and 4) and IL-2 (FIGS. 5 and 6). A sigmoid curve is generated when plotting the log of the **cytokine** standard concentration against the resulting absorbence at 405 nm (OD405) (FIGS. 3 and 5). When the observed absorbence values are. . .

DETDESC:

DETD(88)

Both . . . are validated by performing parallel studies of standards

in serum vs. standards in PBS, and by quantitative recovery of added **cytokines** in serum. Serum parallel experiments are performed by serial dilution of the serum samples which contained immunoreactive IL-1 and IL-2. . .

DETDESC:

DETD(89)

The . . . endogenously in the circulation. This is accomplished by two separate methods. The first study examined is quantitative recovery of the **cytokines** from serum to which known amounts of the **cytokines** has been added ("**cytokine**-spiked serum"). For this assay, standard curves are generated in either standard diluent or human serum diluted 1:1 with assay diluent.

DETDESC:

DETD(90)

Parallel . . . exhibiting a matrix effect, do not contain non-specific factors which inhibit the binding of IL-1 or IL-2 to their respective **antibodies**. Furthermore, the slope and the ED.sub.50 value for both assays (either done in assay diluent or in pre-absorbed serum) are. . .

DETDESC:

DETD(92)

The second approach involves absorption of a **cytokine** by its respective **antibody** and analysis of the absorbed material by high performance liquid chromatography (HPLC). For this study the **antibodies** are diluted 1:500 in coating buffer, and 100 .mu.l of either **antibody** is added to the EIA plate. Twenty-four hours later, the plates are washed with wash buffer and 100 .mu.l of. . .

DETDESC:

DETD(93)

One minute fractions, collected from a linear HPLC gradient separation of the **cytokine** diluted in assay diluent, are tested in their respective EIAs. The resultant immunoactivity is then compared to the immunoreactivity of serum which has been fractionated by HPLC, captured by **antibody** and spiked with the **cytokine** (FIG. 7). IL-1 or IL-2 **antibodies** used in the EIAs are used to capture the IL-1 or IL-2 from the **cytokine**-spiked serum. The results indicate that the material captured on the plate migrated to the same position in the chromatogram as the standard. FIG. 7 demonstrates that the immunoreactivity of **antibody**-absorbed serum is highest in those fractions which co-eluted with the authentic IL-1 or IL-2 standard, although other proteins are detected. . .

DETDESC:

DETD(95)

The IL-1 **antibody**-captured sample was also tested by Western blot analysis. FIG. 8 indicates that a single band of immunoreactive material co-migrates with. . .

DETDESC:

DETD(97)

The . . . serum, containing IL-1 and IL-2 immunoreactivity, shows diminished IL-1 or IL-2 levels following immunoabsorption with either the IL-1 or IL-2 **antibodies**. Prior to the experiment, the sample is shown to contain 2.5 ng/ml IL-1 and 20 ng/ml IL-2. The sample is divided into two aliquots and incubated with either IL-1 or IL-2 **antibody** under standard assay conditions. Subsequently, the sample is removed from the wells and analyzed for IL-1 and IL-2 levels in. . .

DETDESC:

DETD(98)

Internal . . . are calculated to contain 3.1 and 17.5 U/ml, respectively, in the EIA. Each EIA is tested for cross-reactivity with other **cytokines** or serum factors.

DETDESC:

DETD(100)

In each assay, cross-reactivity of the reagents with other **cytokines** and major serum factors is also tested. In this study, each **cytokine** is tested at the highest concentration of standard (100 ng/ml) in each EIA, while the serum factors are tested at concentrations of up to 1 mg/ml. The results are presented as % cross-reactivity of each **cytokine** as calculated by the following equation: ##EQU1##

DETDESC:

DETD(101)

Both the IL-1 and IL-2 EIA demonstrate a very high degree of specificity. Neither assay recognizes any of the other **cytokines** tested to any significant extent, the cross-reactivity ranging from 0 to 0.5% (Table 1). Furthermore, the serum factors tested do. . . the assay at concentrations that exceeded the standard concentration by 100,000-fold. The cross-absorption study demonstrated that the IL-1 and IL-2 **antibodies** specifically recognize their respective **cytokine** in serum. For example, IL-1 levels do not change when the serum sample is treated with the IL-2 **antibody**.

DETDESC:

DETD(102)

Following absorption of the sample with the anti-IL-2 **antibody**, IL-1 levels decrease from 3 to 2.5 ng/ml. In contrast, when the sample is absorbed with the anti-IL-1 **antibody**, the IL-I concentration falls to 1.5 ng/ml. Similarly, absorption with the anti-IL-2 **antibody** decreases measured IL-2 levels from 18 ng/ml to 2 ng/ml, whereas absorption with the anti-IL-1 **antibody** has essentially no effect (IL-2 concentration of 16 ng/ml).

DETDESC:

DETD(103)

TABLE 1

Cross-Reactivity (in %) of **Cytokines** and Other Serum Components with IL-1.alpha. and IL-2 EIAs

Test Substance	IL-1.alpha. EIA	IL-2 EIA

IL-1.alpha.	100	0.0. . .
IgG	0.0	0.0
Hemoglobin .beta.	0.0	0.0
Bilirubin	0.0	0.0
a2-macroglobulin	0.0	0.0

Abbreviations: TNF -- **tumor necrosis** factor; GMCSF: granulocyte/macrophag colony stimulating factor; GCSF: granulocyte colony stimulating factor; MCSF: macrophage colony stimulating factor.

DETDESC:

DETD(107)

Data . . . three normal volunteers indicated that IL-1 and IL-2 levels are not stable throughout the day, with a large number of **cytokine** spikes occurring throughout the day (FIGS. 9-11). The data demonstrate that changes in IL-1 levels are temporally reflected by. . . in IL-2. As previously demonstrated this temporal coincidence is not related to assay cross-reactivity because neither assay detects the alternate **cytokine**.

DETDESC:

DETD(109)

Thus, the **cytokine** levels measured in human serum by the EIA according to the present invention range from 0.5-1.5 ng/ml for IL-1 and 1 to 8 ng/ml for IL-2. It is interesting to note that the **cytokine** levels reported herein are an order of magnitude lower than the reported dissociation constants of their respective **receptors** (Kilian, P. L. et al., J. IMMUNOL. 136:4509-4514 (1986); Dower, S. K. et al., J. EXP. MED. 162:501-515 (1985)). This relationship between **receptor** affinity and circulating hormone concentration is consistent with other well described endocrine systems.

DETDESC:

DETD(112)

Measurement of **Cytokines** in Saliva

DETDESC:

DETD(116)
Measurement of **Cytokines** in Nasal Secretions

DETDESC:

DETD(117)

As . . . a molecular weight of about 60-30 kDa is observed in a Western blot of nasal secretions developed with an anti-IL-2 **antibody**. Pretreatment of the starting material with the anti-IL-2 **antibody** absorbed out this band. However, if the sample is overloaded with IL-2 antigen prior to the absorption step, the band. . .

DETDESC:

DETD(119)
Physical and Psychological Stress Modulates Salivary **Cytokines**

DETDESC:

DETD(120)

The . . . is accompanied by significant rises in IL-1 and IL-2 concentrations. Psychological stress appears to have a greater anticipatory effect, wherein **cytokine** levels increase prior, and in anticipation of, the school examination.

DETDESC:

DETD(122)
Use of Salivary **Cytokine** Levels to Predict Clinical Symptoms

DETDESC:

DETD(123)

A . . . pressor test, known to be associated with increases in catecholamine levels and increases in heart rate. The levels of salivary **cytokines** are determined as above. Salivary IL-1 and IL-2 are elevated both in anticipation of and during the course of stress. These changes in **cytokines** can be correlated with disease symptoms and generate useful predictors for onset of these symptoms.

DETDESC:

DETD(125)
Comparison of **Cytokine** From Saliva With a Standard **Cytokine**

CLAIMS:

CLMS(1)

We claim:

1. A method of monitoring **cytokine** therapy in a human or animal, wherein the **cytokine** is able to bind a carrier molecule but is not IL-1, comprising the steps of
(1) obtaining a sample of body fluid from the human or animal without dissociation of the **cytokine** from bound carrier molecule, wherein the **cytokine** has been administered to the human or animal;
(2) combining the sample of body fluid with,
(a) an **antibody** capable of binding specifically to substantially all of the **cytokine**, whether free or bound to a carrier molecule, wherein the **antibody** is immobilized on a solid phase support, and
(b) a labeled binding epitope of the **cytokine**, wherein the labeled binding epitope competes with the **cytokine** for **antibody** binding, to form an assay mixture;
(3) incubating the assay mixture to allow the immobilized **antibody** to bind specifically to either the **cytokine** or the labeled binding epitope;
(4) washing away unbound labeled binding epitope from the solid phase support;
(5) detecting bound label on the solid phase support;
(6) determining the amount of substantially all of the **cytokine** in the sample; and
(7) comparing the amount of substantially all of the **cytokine** in the sample with a determination of substantially all of the **cytokine** in a previous sample of body fluid.

CLAIMS:

CLMS(2)

2. The method of claim 1, wherein the **cytokine** is selected from the group consisting of **interleukin**-2, **interleukin**-6, **interferon**-.alpha., **interferon**-gamma and **tumor necrosis** factor-.alpha..

CLAIMS:

CLMS(3)

3. The method of claim 2, wherein the **cytokine** is **interleukin**-2.

CLAIMS:

CLMS(4)

4. The method of claim 2, wherein the **cytokine** is **interleukin**-6.

CLAIMS:

CLMS(6)

6. The method of claim 1, wherein the **antibody** is polyclonal.

CLAIMS:

CLMS(7)

7. A method for determining an amount of substantially all of a **cytokine** in a sample of body fluid, wherein the **cytokine** is able to bind a carrier molecule but is not IL-1, comprising the steps of

(1) obtaining the sample of body fluid without dissociation of the **cytokine** from bound carrier molecule;

(2) combining the sample of body fluid with,

(a) an **antibody** capable of binding specifically to substantially all of the **cytokine**, whether free or bound to a carrier molecule, wherein the **antibody** is immobilized on a solid phase support, and

(b) a labeled binding epitope of the **cytokine**, wherein the labeled binding epitope competes with the **cytokine** for **antibody** binding, to form an assay mixture;

(3) incubating the assay mixture to allow the immobilized **antibody** to bind specifically to either the **cytokine** or the labeled binding epitope;

(4) washing away unbound labeled binding epitope from the solid phase support;

(5) detecting bound label on the solid phase support; and

(6) determining the amount of substantially all of the **cytokine** in the sample.

CLAIMS:

CLMS(8)

8. The method of claim 7, wherein the **cytokine** is selected from the group consisting of **interleukin**-2, **interleukin**-6, **interferon**-.alpha., **interferon**-gamma and **tumor necrosis** factor-.alpha..

CLAIMS:

CLMS(9)

9. The method of claim 8, wherein the **cytokine** is **interleukin**-2.

CLAIMS:

CLMS(10)

10. The method of claim 8, wherein the **cytokine** is **interleukin**-6.

CLAIMS:

CLMS(12)

12. The method of claim 7, wherein the **antibody** is polyclonal.